

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
14 October 2004 (14.10.2004)

PCT

(10) International Publication Number
WO 2004/087950 A3

(51) International Patent Classification⁷: **C12Q 1/68**

(21) International Application Number:
PCT/EP2004/003457

(22) International Filing Date: 1 April 2004 (01.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
03007458.7 4 April 2003 (04.04.2003) EP
03014929.8 1 July 2003 (01.07.2003) EP
03017561.6 7 August 2003 (07.08.2003) EP

(71) Applicant (for DE only): **ROCHE DIAGNOSTICS GMBH** [DE/DE]; Sandhofer Strasse 116, 68305 Mannheim (DE).

(71) Applicant (for all designated States except DE): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SAGNER, Gregor**

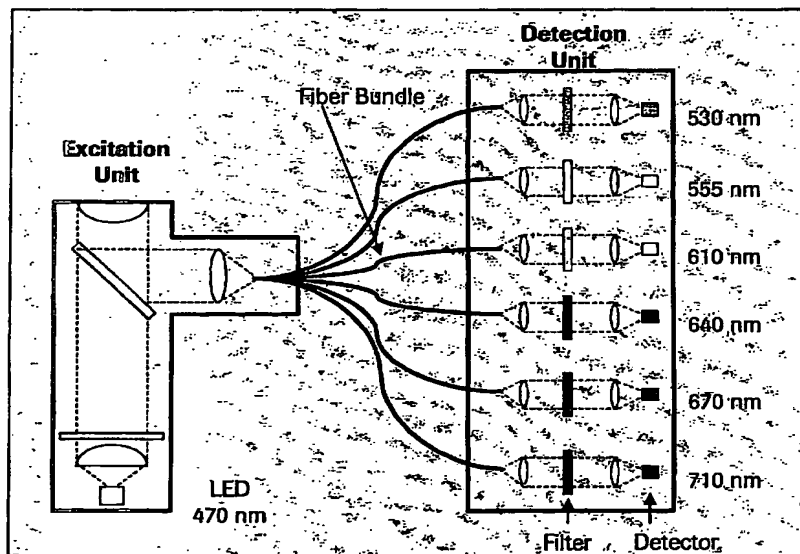
[DE/DE]; Meichelbeckstrasse 16, 82377 Penzberg (DE). **BECHLER, Ingrid** [DE/DE]; Wolfpratshauser Strasse 34 a, 82538 Gelting (DE). **BOLTE, Joachim** [DE/DE]; Martin-Luther Strasse 2c, 68642 Buerstadt (DE). **HEINDL, Dieter** [DE/DE]; Sternstrasse 4, 82396 Paehl (DE). **JOSEL, Hans-Peter** [DE/DE]; Ulmenstrasse 28, 82362 Weilheim (DE). **GUTEKUNST, Martin** [DE/DE]; Escherstrasse 12, 82390 Eberfing (DE). **SEIBL, Rudolf** [DE/DE]; Kapellenwiese 29, 82377 Penzberg (DE). **MUELLER, Christoph** [DE/DE]; Ambacher Strasse 19, 81476 Muenchen (DE).

(74) Common Representative: **ROCHE DIAGNOSTICS GMBH**; C/O Heiko Hillebrandt, 82377 Penzberg (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: **IMPROVED SYSTEM FOR MULTI COLOR REAL TIME PCR**



(57) Abstract: The invention is directed to a system for performing multi-color real time PCT, comprising a flexible real time PCT instrument and a specific composition or reaction mixture for performing multiplex PCR: In particular, the present invention is directed to a composition or reaction mixture which comprises at least 3, preferably 4-5 and most preferably exactly 4 pairs of FRET hybridization probes. Each pair of said hybridization probes consists of a FRET donor probe carrying a FRET donor moiety and a FRET acceptor probe carrying a FRET acceptor moiety having an emission maximum between 550 and 710 nm.

WO 2004/087950 A3



- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ,

SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- of inventorship (Rule 4.17(iv)) for US only

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:

25 November 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/003457

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 6 150 107 A (MATHIES RICHARD A ET AL) 21 November 2000 (2000-11-21) abstract column 2, line 1 - column 2, line 28 examples 1-5 claims 1-28 figures 1-8 ----- -/--	1-9, 13, 14 10-12, 15-17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

30 September 2004

Date of mailing of the international search report

13/10/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Madlener, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003457

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 6 177 247 B1 (JU JINGYUE ET AL) 23 January 2001 (2001-01-23) abstract column 2, line 11 - column 2, line 34 column 3, line 46 - column 4, line 16 column 5, line 32 - column 5, line 45 column 6, line 13 - column 6, line 22 column 7, line 18 - column 7, line 21 example 1 claims 1-45	1-9, 13, 14 10-12, 15-17
X Y	US 5 869 255 A (JU JINGYUE ET AL) 9 February 1999 (1999-02-09) abstract claims 1-20 figures 1,3	1-9, 13, 14 10-12, 15-17
X Y	VET J A M ET AL: "Multiplex detection of four pathogenic retroviruses using molecular beacons" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 96, 1999, pages 6394-6399, XP002145609 ISSN: 0027-8424 abstract page 6339, left-hand column figures 1,2	1-9, 13, 14 10-12, 15-17
X Y	JU J ET AL: "DESIGN AND SYNTHESIS OF FLUORESCENCE ENERGY TRANSFER DYE-LABELED PRIMERS AND THEIR APPLICATION FOR DNA SEQUENCING AND ANALYSIS" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 231, no. 1, 10 October 1995 (1995-10-10), pages 131-140, XP000536518 ISSN: 0003-2697 abstract figures 1,3	1-9, 13, 14 10-12, 15-17
	----- -/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003457

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Rotor-Gene 3000 Four-Channel Multiplexing System" ANNOUNCEMENT CORBETT RESEARCH, XX, XX, 2002, pages 1-8, XP002264359	15-17
Y	the whole document	1-14
Y	EP 0 640 828 A (HOFFMANN LA ROCHE) 1 March 1995 (1995-03-01) the whole document	1-17
Y	US 6 369 893 B1 (CHANG RONALD ET AL) 9 April 2002 (2002-04-09) abstract column 3, line 41 - column 5, line 42	1-17
Y	WO 97/46714 A (RASMUSSEN RANDY P ; UNIV UTAH RES FOUND (US); RIRIE KIRK M (US); WITTW) 11 December 1997 (1997-12-11) abstract page 4, paragraph 3 - page 22 examples 5-9 claims 1-126	1-17
Y	US 6 197 520 B1 (BERNARD PHILIP ET AL) 6 March 2001 (2001-03-06) the whole document	1-17
Y	WO 98/49340 A (CORBETT JOHN MICHAEL JR ; CORBETT JOHN MICHAEL (AU)) 5 November 1998 (1998-11-05) the whole document	1-17
Y	PALLADINO SILVANO ET AL: "Real-time PCR for the rapid detection of vanA and vanB genes." DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE. JAN 2003, vol. 45, no. 1, January 2003 (2003-01), pages 81-84, XP002298326 ISSN: 0732-8893 the whole document	1-17
A	MATTHEWS J A ET AL: "ANALYTICAL STRATEGIES FOR THE USE OF DNA PROBES" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, NEW YORK, NY, US, vol. 169, no. 1, 15 February 1988 (1988-02-15), pages 1-25, XP000670180 ISSN: 0003-2697 the whole document	1-17

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/003457

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>MARRAS SALVATORE A E ET AL: "Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes." NUCLEIC ACIDS RESEARCH. 1 NOV 2002, vol. 30, no. 21, 1 November 2002 (2002-11-01), page e122, XP002298327 ISSN: 1362-4962 the whole document</p> <p>-----</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2004/003457

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☒ contained in the International application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/003457

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6150107	A	21-11-2000	US 5853992 A	29-12-1998
			AU 717018 B2	16-03-2000
			AU 4742397 A	24-04-1998
			CA 2267654 A1	09-04-1998
			EP 0935670 A1	18-08-1999
			GB 2317951 A ,B	08-04-1998
			JP 2001502000 T	13-02-2001
			WO 9814612 A1	09-04-1998
US 6177247	B1	23-01-2001	US 5869255 A	09-02-1999
			US 5654419 A	05-08-1997
			US 2003143594 A1	31-07-2003
			US 6028190 A	22-02-2000
			US 6544744 B1	08-04-2003
			AT 236994 T	15-04-2003
			AU 692230 B2	04-06-1998
			AU 1736795 A	21-08-1995
			CA 2182516 A1	10-08-1995
			DE 19581489 B4	15-07-2004
			DE 19581489 T0	02-01-1997
			DE 29521620 U1	13-11-1997
			DE 69530286 D1	15-05-2003
			DE 69530286 T2	01-04-2004
			EP 0743987 A1	27-11-1996
			ES 2197193 T3	01-01-2004
			JP 9508525 T	02-09-1997
			WO 9521266 A1	10-08-1995
			US 5707804 A	13-01-1998
			US 5688648 A	18-11-1997
US 5869255	A	09-02-1999	US 5654419 A	05-08-1997
			US 6177247 B1	23-01-2001
			US 2003143594 A1	31-07-2003
			US 6028190 A	22-02-2000
			US 6544744 B1	08-04-2003
			AT 236994 T	15-04-2003
			AU 692230 B2	04-06-1998
			AU 1736795 A	21-08-1995
			CA 2182516 A1	10-08-1995
			DE 19581489 B4	15-07-2004
			DE 19581489 T0	02-01-1997
			DE 29521620 U1	13-11-1997
			DE 69530286 D1	15-05-2003
			DE 69530286 T2	01-04-2004
			EP 0743987 A1	27-11-1996
			ES 2197193 T3	01-01-2004
			JP 9508525 T	02-09-1997
			WO 9521266 A1	10-08-1995
			US 5707804 A	13-01-1998
			US 5688648 A	18-11-1997
EP 0640828	A	01-03-1995	AT 192851 T	15-05-2000
			AU 681682 B2	04-09-1997
			AU 7141494 A	09-03-1995
			BR 9403338 A	11-04-1995
			CA 2129787 A1	28-02-1995
			CN 1107892 A ,B	06-09-1995
			CZ 9402078 A3	15-11-1995

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/EP2004/003457

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0640828	A	DE 69424353 D1	15-06-2000
		DE 69424353 T2	04-01-2001
		DK 640828 T3	25-09-2000
		EP 0640828 A1	01-03-1995
		ES 2147565 T3	16-09-2000
		FI 943936 A	28-02-1995
		HU 71622 A2	29-01-1996
		IL 110732 A	15-07-1998
		JP 7163397 A	27-06-1995
		NO 943166 A	28-02-1995
		NZ 264310 A	25-03-1998
		PL 304805 A1	06-03-1995
		SG 47865 A1	17-04-1998
		ZA 9406330 A	28-02-1995
US 6369893	B1	09-04-2002	
		US 6565815 B1	20-05-2003
		US 2002109844 A1	15-08-2002
		AT 228242 T	15-12-2002
		AU 746069 B2	11-04-2002
		AU 4194099 A	06-12-1999
		CA 2331678 A1	25-11-1999
		DE 69904043 D1	02-01-2003
		DE 69904043 T2	31-07-2003
		EP 1080364 A1	07-03-2001
		ES 2183563 T3	16-03-2003
		JP 2002515602 T	28-05-2002
		WO 9960380 A1	25-11-1999
		US 2003221771 A1	04-12-2003
		US 2003152492 A1	14-08-2003
WO 9746714	A	11-12-1997	
		AT 260988 T	15-03-2004
		AU 727296 B2	07-12-2000
		AU 3154797 A	05-01-1998
		AU 729644 B2	08-02-2001
		AU 3380097 A	05-01-1998
		AU 726501 B2	09-11-2000
		AU 3481297 A	05-01-1998
		CA 2256612 A1	11-12-1997
		CA 2256773 A1	11-12-1997
		CA 2257109 A1	11-12-1997
		DE 69727932 D1	08-04-2004
		EP 1179600 A1	13-02-2002
		EP 1442794 A2	04-08-2004
		EP 0912760 A2	06-05-1999
		EP 0906449 A2	07-04-1999
		EP 0912766 A1	06-05-1999
		EP 1033411 A2	06-09-2000
		JP 2000512138 T	19-09-2000
		JP 2000511435 T	05-09-2000
		JP 2000509608 T	02-08-2000
		KR 2000016161 A	25-03-2000
		KR 2000016326 A	25-03-2000
		NZ 333135 A	28-02-2000
		NZ 333136 A	27-03-2000
		NZ 333137 A	27-03-2000
		NZ 502323 A	28-09-2001
		WO 9746707 A2	11-12-1997
		WO 9746712 A2	11-12-1997

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003457

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9746714	A		WO 9746714 A1	11-12-1997
			US 2004002098 A1	01-01-2004
			US 6245514 B1	12-06-2001
			US 6232079 B1	15-05-2001
			US 2002058258 A1	16-05-2002
			US 6174670 B1	16-01-2001
<hr/>				
US 6197520	B1	06-03-2001	AT 239095 T	15-05-2003
			AU 6635500 A	13-03-2001
			DE 60002424 D1	05-06-2003
			DE 60002424 T2	18-03-2004
			EP 1121459 A2	08-08-2001
			ES 2199172 T3	16-02-2004
			JP 2003507696 T	25-02-2003
			WO 0112854 A2	22-02-2001
<hr/>				
WO 9849340	A	05-11-1998	AU 7013698 A	24-11-1998
			WO 9849340 A1	05-11-1998
<hr/>				